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MONOCYTE CHEMOTAXIS MEDIATED BY FORMYL-METHIONYL-LEUCYL-PHENYLALANINE CONJUGATED WITH MONOCLONAL ANTIBODIES AGAINST HUMAN OVARIAN CARCINOMA

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Abstract—Availability of a chemically defined chemoattractant (fMLP) and of appropriate monoclonal antibodies may permit local manipulation of the inflammatory response to human tumors. fMLP has been conjugated with two monoclonal antibodies (OC125 and OC133) which react with human ovarian carcinomas. Conjugates retained the ability to bind to a human ovarian carcinoma line (OVCA433) judged by indirect immunofluorescence and by radioimmunossay. The fMLP conjugate was maximally chemotactic for human blood monocytes and human peritoneal macrophages at protein concentrations of 300–900 μg/ml. Conjugates stimulated chemotaxis rather than chemokinesis. After incubation with an fMLP-antibody conjugate, antigen positive OVCA433 cells released chemotactic activity and attracted monocytes *in vitro*, whereas an antigen-negative ovarian cell line failed to do so. As monocytes can be important effectors of antibody dependent cell mediated cytoxicity, fMLP conjugates might increase monocyte concentrations at tumor sites and potentiate serotherapy for certain human neoplasms.

A number of studies have documented the ability of macrophages to destroy tumor cells *in vitro* either in the presence or absence of specific antibody (Nathan, Murray & Cohn, 1980). The importance of macrophages as effector cells in the host's defense against tumors *in vivo* still remains to be established, although there is substantial evidence that macrophages contribute to the antitumor activity of certain immunostimulants including Bacillus Calmette-Guérin and *Corynebacterium parvum* (Bast, Bast & Rapp, 1976; Nathan *et al.*, 1980). To eliminate tumor cells, macrophages must first migrate to the site of tumor growth. In addition, macrophages must be "activated" in order to kill tumor cells. Activation of macrophages can result from treatment with lymphokines, interferons and endotoxins as well as certain drugs. Once activated, macrophages can kill tumor cells with which they come in direct contact, possibly through the release of highly reactive oxygen species or proteolytic enzymes.

Formylated oligopeptides, including formyl-methionyl-leucyl-phenylalanine (fMLP), are chemotactic for inflammatory cells. Moreover, fMLP can stimulate release of reactive oxygen species. Previous studies have demonstrated that fMLP can be covalently coupled to heteroantibodies, forming conjugates that are chemotactic and that retain the ability to bind specifically to antigen (Isturiz, Sandberg, Schiffmann, Wahl & Notkins, 1978). Our own recent studies have shown that fMLP can be conjugated to a polyclonal rabbit IgG reactive with a guinea pig hepatoma (Obrist & Sandberg, 1982). Immunoglobulin-fMLP conjugates retain their ability to bind to antigen, attract monocytes and stimulate O_2^- release from guinea pig peritoneal cells *in vitro* (Obrist & Sandberg, 1982). Modifi d antibodies can also attract macrophages into tumors *in vivo* (Sandberg, Obrist & Mergenhagen, 1982), but it has been difficult to obtain heteroantibody in sufficient amounts to perform extensive studies either in animal models or in clinical trials. Given the recent development of monoclonal antibodies reactive with human tumors (Mitchell & Oettgen, 1982), we have evaluated the feasibility of coupling fMLP to murine monoclonal immunoglobulins which bind to human ovarian carcinomas (Bast, Feeney, Lazarus, Nadler, Colvin & Knapp, 1981). Conjugated immunoglobulins have been tested for

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their ability to bind epithelial ovarian carcinoma cell lines and for their ability to stimulate chemotaxis of human monocytes and macrophag s.

EXPERIMENTAL PROCEDURES

Antibody preparation

Preparation and characterization of monoclonal antibodies reactive with human ovarian carcinoma has been described (Bast *et al.*, 1981; Kabawat, Bast, Welch, Knapp & Colvin, 1983; Kabawat, Bast, Bhan, Welch, Knapp & Colvin, 1983; Masuho, Zalutsky, Knapp & Bast, in preparation). In brief, OC125 recognizes a Mullerian differentiation antigen that is expressed by a majority of epithelial ovarian carcinomas and adenomas of non-mucinous histology (Bast *et al.*, 1981; Kabawat *et al.*, 1983). When judged by indirect immunofluorescence, OC125 failed to react with a variety of normal adult tissues (Bast *et al.*, 1981). Using the more sensitive biotin-avidin immunoperoxidase technique, OC125 exhibited slight, but definite reactivity with epithelial components of the adult fallopian tube, endometrium and endocervix (Kabawat *et al.*, 1983). Among fetal tissues, OC125 bound to pleural and peritoneal mesothelium in addition to Mullerian duct derivatives, but reactivity with pleura and peritoneum was not found in the adult (Kabawat *et al.*, submitted). OC133 reacts with serous cystadenocarcinomas of the ovary as well as with adult endometrium and endocervix (Berkowitz, Kabawat, Lazarus, Colvin, Knapp & Bast, in press). Despite restricted reactivity with nonmalignant adult tissues, OC133 reacted with 5 of 6 nonovarian tumor cell lines whereas OC125 reacted with only 1 of 14 nonovarian tumor cell lines (Bast *et al.*, 1981). Immunochemical studies suggest that OC125 recognizes epitopes associated with a glycoprotein of >500,000 daltons, whereas OC133 reacts with a distinct moiety of 80,000 daltons (Masuho *et al.*, in preparation).

Clones OC125 and OC133 were passaged intraperitoneally in pristane primed BALB/c mice (Jackson Laboratories, Bar Harbor, Maine). Ascites was harvested one to two weeks following the i.p. injection of 10^6 hybridoma cells. All cellular components were removed by centrifugation at 1000 *g* for 10 min at 20°C. OC133 was precipitated with $(NH4)_2SO_4$ at 50% saturation and subsequently dialysed exhaustively against 0.9% NaCl until no precipitation occurred with 1% BaCl. In experiments with OC133, IgG was further purified by DE52 (Whatman Biochemicals, Springfield Mill, Maidstone, Kent, England) ion exchange chromatography eluting with a linear NaCl gradient from 0 to 0.3 M. Fractions were concentrated by ultrafiltration (Amicon Corp., Lexington, Massachusetts), the presenc of IgG was verified by precipitation with goat anti-mouse IgG (Cappel Lab. Inc., Cochranville, Pennysylvania) in agarose gel and the protein concentration was estimated according to Lowry, Rosebrough, Farr & Randall (1951). To prepare OC125-fMLP conjugates, immunoglobulin from the ammonium sulfate precipitation was coupled with fMLP.

Coupling to fMLP

fMLP (Sigma Chemical Co., St. Louis, Missouri), monoclonal antibody and 1-cyclohexyl-3-(2-morpholinoethyl) carbodiimide metho-*p*-toluenesulfonate (CDI) (Aldrich Chemical Co., Inc., Milwaukee, Wisconsin) were mixed in a 1:3.6:36.3 ratio by weight, incubated for 20 min at room temperature and dialysed extensively against Dulbecco's phosphate buffered saline (PBS) (No. 310-4080; Grand Island Biological Company, Grand Island, New York). In pilot experiments we determined an optimal protein concentration in the range of 1–3 mg/ml. With higher concentrations of immunoglobulin, most of the protein precipitated during the reaction. Conjugates and control preparations containing antibody alone or antibody reacted with CDI without fMLP were further purified as described.

Flow cytometry

Analysis of immunoglobulin binding using flow cytometry has been described in detail (Bast *et al.*, 1981). Antibody binding was evaluated by indirect immunofluorescence using the OVCA433 epithelial ovarian carcinoma cell line as a target. After incubation with monoclonal antibody target cells were washed 3 times and incubated with fluorescein conjugated goat anti-mouse immunoglobulin (Cappel Lab., Cochranville, PA) as fluorophore. After 3 additional washings, fluorescence associated with tumor cells was evaluated in a L30 cytoflurograph (Ortho Instruments, Westwood, Massachusetts).

Radioimmunoassay

During radioimmunoassays all incubations were performed at 0°C. OVCA433 cells (2×10^5) were incubated for 30 min with antibody or antibody conjugate in 50 μl PBS with 5% heat inactivated fetal bovine serum. Tumor cells were washed three times and incubated for another 30 min with 50 μl of ^{125}I-coupled anti-mouse IgG (New England Nuclear, Boston, Massachusetts). After three washes radioactivity associated with the cells was measured in a gamma counter.

Cells for chemotaxis assays

Peripheral blood mononuclear cells were isolated from heparinized venous blood by Ficoll/Hypaque density gradient centrifugation (LSM, Litton Bionetics, Kensington, Maryland), and washed in Hanks' balanced salt solution (BSS). Mononuclear leukocytes were counted in a hemacytometer and cytological preparations were sedimented by centrifugation (Cytospin, Shandon Southern Products Ltd., Astmoor, Runcorn, Cheshire, England). Cytological preparations were stained with Diff-Quick® (Harleco, Gibbstown, New Jersey) and with a stain for nonspecific esterase (Koski, Poplack & Blaese, 1976). The cell suspension was adjusted to a concentration of 2.5×10^6 cells/ml in Dulbecco's Modified Eagle Medium with 4.5 g/l glucose (M.A. Bioproducts, Walkersville, Maryland) supplemented with 2% bovine serum albumin (Miles Laboratories, Inc., Elkhart, Indiana), 2 mM L-glutamine, 10 mM HEPES and 1 mM sodium pyruvate (M.A. Bioproducts, Walkersville, Maryland).

Using protocols approved by the Human Studies Committee of the Brigham and Women's Hospital and the Sidney Farber Cancer Institute, peritoneal macrophages were obtained from patients who underwent laparoscopy for diagnosis of gynecological conditions or for tubal ligation. Briefly, the peritoneum was filled with CO_2, the pelvic cul-de-sac was visualized to exclude adhesions or obstruction. A total of 1 l 0.9% saline was then washed into the peritoneal cavity through the laparoscope. The saline was removed by culdocentesis into an Inpersol® peritoneal dialysis container (Abbott Laboratories, North Chicago, Illinois). Peritoneal cells were separated from lavage fluid by centrifugation at 320 *g* for 20 min in 250 ml polypropylene bottles (Corning Glass Works, Corning, New York). The cell pellet was resuspended in Hanks' BSS. Both leukocytes and erythrocytes were enumerated in a hemocytometer. Erythrocyte counts in peritoneal washings were compared to erythrocyte counts in peripheral blood. The volume of peripheral blood which contaminated the peritoneal washings was calculated based upon the assumption that all of the erythrocytes present in the peritoneal washings had been contributed by peripheral blood introduced during insertion of the laparoscope. Peritoneal cell suspensions in which more than 2% of the leukocytes could have been contributed by peripheral blood were discarded. Monocyte levels were calculated from the total cell count, differential counts of stained smears and the proportion of esterase positive cells. Cell concentrations were adjusted to 2.5×10^6 monocytes/ml.

Chemotaxis assays

The chemotaxis assay was performed as described previously (Falk, Goodwin & Leonard, 1980; Obrist & Sandberg, 1982) using a microchemotaxis chamber (Neuro Probe, Inc., Bethesda, Maryland), 2.5×10^6 monocytes or macrophages/ml and 90 min incubation at 37°C in 5% CO_2.

RESULTS

Chemotaxis mediated by antibody-fMLP conjugates in solution

Antibody conjugates were tested for their ability to attract human peripheral blood mononuclear (PBM) cells. PBM chemotaxis was evaluated at different concentrations of OC125, OC125-fMLP or free fMLP (Table 1). Non-conjugated OC125 lacked chemotactic activity over a broad range of concentration (30–1000 μg/ml), whereas 30 μg/ml of the OC125-fMLP conjugate increased the accumulation of PBM cells 3-fold. Maximal chemotactic activity was observed with 300 μg/ml of the conjugate. When free fMLP was tested, optimal chemotactic activity was produced at 10^{-8} M.

To rule out the possibility that antibody-fMLP conjugates stimulated nonspecific chemokinesis rather than the directed migration of leukocytes, concentrations of the conjugates were varied on either side of the membrane barrier that divided the chemotaxis chamber. In this setting, a stimulator of chemokinesis would increase the number of migrating cells when placed in either compartment. A true chemoattractant would promote migration of cells only in one direction along a gradient of increasing antibody-fMLP concentrations. Judging from the data in Table 2, an OC133-fMLP conjugate stimulated true chemotaxis rather than chemokinesis. High concentrations of the conjugate in the compartment primed with cells failed to stimulate migration into the other compartment. Similar results have been obtained with OC125-fMLP conjugates (data not shown).

If antibody-fMLP conjugates are to be used to treat human ovarian carcinoma, it is important that they be capable of attracting peritoneal macrophages as well as PBM cells. Human PC chemotaxis was evaluated with cells from three patients who underwent laparoscopy for primary infertility. None of the patients had active pelvic inflammatory disease. The response of PBM and PC from the same individuals were compared in the presence of different concentrations of antibody or antibody-fMLP conjugates (Table 3). Among the concentrations tested, optimal chemotaxis was observed for both cell types in the

Table 1. Chemotactic response of human peripheral blood mononuclear cells to an antibody-fMLP conjugate, antibody alone or free fMLP*

Chemoattractant	Concentration	Mononuclear response (cells/hpf)
Medium		21.2± 4.0
OC125-fMLP	1000 μg/ml	106.7±11.0
	300	116.3± 1.8
	100	79.1± 5.4
	30	66.3± 0.4
OC125	1000 μg/ml	17.1± 1.8
	300	15.8± 3.1
	100	17.6± 1.6
	30	16.6± 3.1
fMLP	1010^{-7} M	72.2± 9.4
	10^{-8}	86.7±13.8
	10^{-9}	58.1± 6.4
	10^{-10}	31.9± 2.3

* Chemotaxis of human peripheral blood monocytes was assayed in a modified Boyden chamber microsystem. The assay was run with 50 μl of 2.5×10^{-6} ml esterase positive cells for 90 min at 37°C. 10 high per fields (1000×) were counted per filter, averaged and the mean ± S.E.M. of triplicate filters calculated.

Table 2. Chemotactic response of human peripheral blood mononuclear cells to an antibody-fMLP conjugate in the same or a different chamber

	OC133-fMLP (μg/ml) Upper chamber			
	900	300	100	0
OC133-fMLP (μg/ml) Lower chamber				
900	13.7±2.7	25.1±3.9	32.6±2.4	47.2±4.2
300	6.4±0.9	9.7±2.3	24.1±3.1	41.4±1.8
100	4.0±0.4	4.4±0.8	13.6±1.1	31.4±3.1
0	2.0±0.5	1.9±0.2	3.0±0.5	3.1±3.1

Assay of OC133 conjugated fMLP with human peripheral blood monocytes under the same conditions as in Table 1. PBL's were placed in the upper chamber and migration measured in the lower chamber.

presence of 400 μg/ml of the conjugate. Non-conjugated antibody once again lacked chemotactic activity. Given the amount of variation between patients, no significant difference could be demonstrated in the reactivity of PBM and PC, although PC were slightly less responsive than PBM to either antibody-fMLP conjugates or free fMLP.

Binding of antibody-fMLP conjugates to tumor associated antigens

Conjugation of murine monoclonal immunoglobulins with fMLP might affect the ability of antibodies to bind to tumor associated antigen(s). Consequ ntly, we have compared binding of chemically modified antibody to that of the native immunoglobulin using a radioimmunoassay and indirect immunofluorescence. For both assays OVCA433 cells were incubated with antibody or antibody-fMLP conjugates and washed free from excess reagent. For the radioimmunoassay, cells were then incubated with ^{125}I-labeled rabbit anti-mouse immunoglobulin. Similar binding of radiolabel was observed after preincubation with the antibody-fMLP conjugate or the free antibody (Table 4). For assays of indirect immunofluorescence, cells that had been preincubated with antibody or antibody-fMLP conjugate were incubated with fluoresceinated goat anti-mouse IgG. When fluorescence intensity was

Table 3. Chemotactic response of human peritoneal macrophages and of peripheral blood mononuclear cells to antibody-fMLP conjugates, free fMLP or antibody alone*

Chemoattractant	Concentration	Response of peritoneal macrophages			Response of peripheral blood mononuclear cells		
		Pt. no. 1 (OC133-fMLP)	Pt. no. 2 (OC133-fMLP)	Pt. no. 3 (OC125-fMLP)	Pt. no. 1 (OC133-fMLP)	Pt. no. 2 (OC133-fMLP)	Pt. no. 3 (OC125-fMLP)
Medium		2.6	17.1	3.1	16.1	10.3	10.2
Antibody-fMLP	400 μg/ml	14.9	96.9	58.4	110.4	50.0	36.2
	100	25.2	58.3	31.6	81.2	40.2	29.1
	25	5.9	39.8	22.8	47.6	13.8	17.9
fMLP	10^{-7} M	19.5	50.5	19.1	57.1	32.0	25.8
Antibody	400 μg/ml	3.0	22.0	4.8	21.2	23.0	9.2
	100	2.2	20.7	4.1	20.6	17.6	9.4
	25	2.4	20.1	2.6	18.9	15.0	7.4

* Each point is the mean of 10 high power fields.

Table 4. Binding of antibody and antibody-fMLP conjugates to ovarian tumor cell targets judged by a radiolabeled anti-globulin assay

Agent	Counts/min ± S.D.
PBS	345± 27
OC125	1214±134
OC125-fMLP	1346±200
OC133	1409±28
OPC133-fMLP	1404±169

The binding of native and conjugated antibody to the ovarian carcinoma cell line OVCA433 was determined by radioimmunoassay. 2×10^{-5} OVCA433 cells were incubated with 50 μl of control medium, antibody or antibody-fMLP conjugate for 30 min, washed (3×) and incubated for another 30 min with 50 μl of [^{125}I]anti-mouse IgG. After 3 additional washes, radioactivity associated with cells was measured in a gamma counter. Results are expressed as mean counts/min ± S.D. of duplicate determinations.

measured for 10,000 individual cells using a flow cytometer, a similar profile of reactivity was obtained with antibody and antibody-fMLP conjugates (Fig. 1).

Chemotaxis mediated by antibody-fMLP conjugates bound to cells

A crucial requirement for the utility of antibody-fMLP conjugates *in vivo* is that the conjugate once bound to the tumor cell surface can be released, establishing a gradient of chemotactic activity. If the conjugate remained firmly bound to the tumor cell surface or was endocytosed, no chemotactic gradient would be established around the tumor cell. To evaluate this possibility OVCA433 cells were incubated with antibody-fMLP conjugates, free antibody or free fMLP. After repeated washing, tumor cells were used as a "chemoattractant".

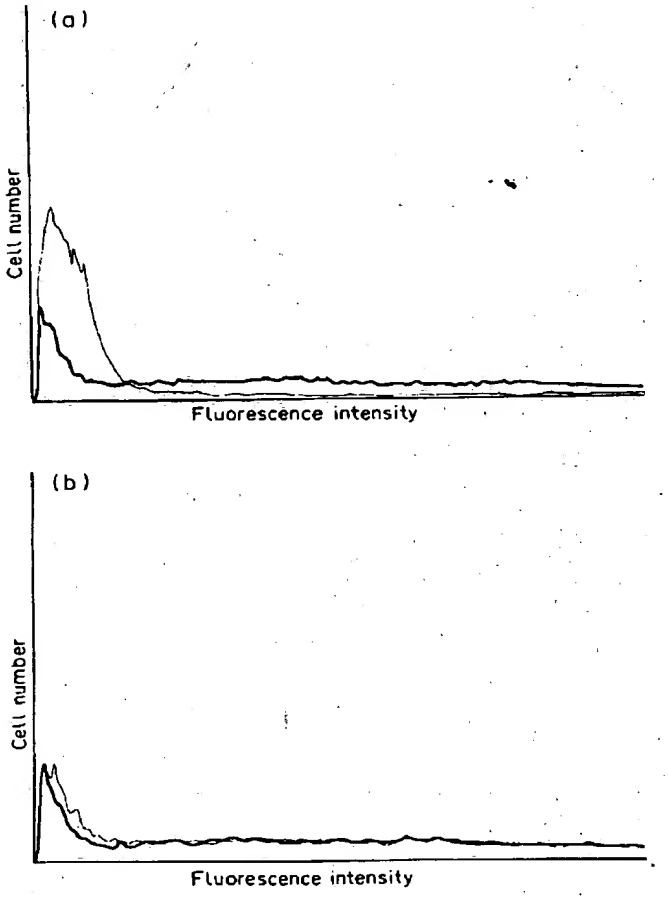

Fig. 1. Cytofluorographic examination of OVCA433 cells, which were first incubated with PBS, OC125 of OC125-fMLP detectable at a dilution that would result in antibody binding to 70% of the cells. Fluoresceinated goat anti-mouse IgG was used in a second incubation. Figure (a) compares the PBS control (----) with the OC125 stained cells (= = = = =). Figure (b) compares OC125(----) with OC125-fMLP (= = = = =) stained cells.

Chemotactic activity was only observed after preincubation with the antibody-fMLP conjugate (Table 5). No activity was observed when cells were incubated with fMLP or antibody alone. Specific binding of the conjugate was required. Incubation of the antibody-fMLP conjugate with a putatively nonmalignant ovarian cell line W434 that lacked reactivity with OC133, failed to impart chemotactic activity.

Table 5. Chemotactic response to human peripheral blood mononuclear cells to antibody-fMLP conjugates associated with ovarian tumor targets

Chemotactic agent	Mononuclear response (cells/hpf)
Medium	12.5±3.5
fMLP 10^{-7} M	50.2±9.6
OVCA433 + PBS	11.7±3.1
OVCA433 + OC133	11.2±0.9
OVCA433 + OC133-fMLP	33.4±2.6
OVCA433 + fMLP	9.7±2.1
W434 + OC133-fMLP	11.0±3.1

In order to test whether a chemotactic gradient is established around tumor cells covered with IgG-fMLP, human peripheral blood monocyte chemotaxis was tested using antigen positive OVCA433 and antigen negative W434 cells as chemoattractant in the lower compartment of the chemotaxis chamber. Before the assay cells were preincubated for 30 mins with PBS, OC133, OC133-fMLP or fMLP alone and then washed exhaustively. Assay conditions are otherwise as in Table 1.

DISCUSSION

The monoclonal mouse antibodies OC125 and OC133, which bind to human epithelial ovarian carcinomas, were covalently coupled with the chemotactic peptide fMLP. These novel compounds induced dose dependent chemotaxis of human PBM and PC *in vitro*, whereas native antibody did not. Migration was due to chemotaxis rather than chemokinesis. Similar results were obtained with conjugates prepared from different murine monoclonal antibodies directed against distinct antigens associated with epithelial ovarian carcinomas. Polyclonal rabbit heteroantibodies acquire chemotactic activity when conjugated with fMLP (Obrist & Sandberg, 1982), but this might not be true for all murine monoclonal immunoglobulins. Certain hybridomas could produce immunoglobulins with few potential conjugation sites. In the present study, however, chemotactic conjugates could be prepared from both the antibodies tested. Conjugation with fMLP also failed to affect antigen recognition function judged by indirect immunofluorescence or radioimmunoassay. CDI might have denatured the antibody or fMLP could have bound near the antigen recognition site. Studies with larger numbers of monoclonal reagents will be required to determine how widely applicable CDI-mediated conjugation with fMLP will be.

Ovarian carcinoma is an unusual neoplasm in that tumor cells metastasize through the peritoneal cavity and implant on the serosal surface of the parietal and visceral peritoneum. Intraabdominal chemothereapy (Speyer, Collins, Dedrick, Brennan, Buckpitt, Londer, DeVita & Meyers, 1980) and immunotherapy (Webb, Oaten & Pike, 1978; Mantovani, Sessa, Peri, Allavona, Introna, Polentarutti & Mangioni, 1981; Bast, Berek, Obrist, Griffiths, Berlowitz, Hacker Parker Lagasse & Knapp, 1983) have produced resolution of ascites and regression of small tumor nodules that had failed to respond to intravenous chemotherapy. Studies with a murine model suggest that ovarian tumors might be susceptible to intraperitoneal serotherapy (Order, Donahue & Knapp, 1973; Order, Kirkman & Knapp, 1974; Bast, Knapp, Donahue, Thurston, Mitchell, Fenney & Schlossman, 1980). A combination of heteroantiserum and the immunostimulant *C. parvum* has proven more effective in inhibiting murine ovarian tumor growth than has either single agent (Knapp & Berkowitz, 1977; Bast, Knapp, Mitchell, Thurston, Tucker & Schlossman, 1979). Doses, routes and schedules of *C. parvum* administration that attract and activate peritoneal macrophages for antibody dependent cell mediated cytotoxicity *in vitro* also augment serotherapy *in vivo*. Silica can eliminate effector function for ADCC *in vitro* and can abrogate the antitumor activity of heteroantiserum and *C. parvum in vivo*. In studies with the murine ovarian carcinoma, as well as other models of serotherapy, the accumulation of appropriately activated macrophages and "K" cells may be a critical requirement for effective immunotherapy with polyclonal or monoclonal reagents.

Intraperitoneal administration of antibody-fMLP conjugates might favor the accumulation of macrophages within ovarian tumor nodules that stud serosal surfaces. Rapid infusion and withdrawal might avoid the "paralysis" of peripheral blood mononuclear cells that is likely to follow intravenous injection of conjugates (Yasak, Boxer & Bachner, 1982). Excess antibody could be lavaged from the peritoneal cavity after binding to tumor had occurred, encouraging the development of a concentration gradient around tumor cells. The observation that chemotactic conjugates once bound to ovarian tumor cells can still be released, establishing a gradient of chemotactic activity, is particularly important. Different results might be

anticipated with antigens that modulate, such as the common acute lymphoblastic leukemia antigen (Ritz, Pesando, Notis-McConarty & Schlossman, 1980). Similar *in vitro* studies will be required with each new antibody-fMLP conjugate before *in vivo* studies can be undertaken.

Both PBM and PC responded to antibody-fMLP conjugates. PBM were slightly more responsive than PC, but a statistically significant difference was not observed. Although studies with larger numbers of donors might document a subtle difference between cells from the two sites, it appears that antibody fMLP conjugates could attract mononuclear cells either from the peripheral blood or from the peritoneal cavity. Our current report concerns the interaction of antibody-fMLP conjugates with mononuclear cells from normal donors. It remains to be shown that these conjugates will mediate migration of similar populations from ovarian cancer patients. Migration of monocytes into the peritoneal cavity in response to an inflammatory agent is defective in rodents with tumors as is the *in vitro* chemotaxis of cells from such animals (Snyderman, Pike & Altman, 1975). Suppression of macrophage migration is due to soluble factors which can be found in sera from tumor bearing hosts or in supernatants from tumor cell lines (Pike & Snyderman, 1976; Snyderman & Pike, 1976). Effects of these factors have been demonstrated *in vitro* by a reduction in the number of cells required for progressive tumor growth or a decreased tumor growth rate in animals treated with partially purified preparations (Pike & Snyderman, 1976). In patients with cancer the *in vivo* migration of macrophages as measured with Rebuck skin windows is also impaired (Israel, Samak, Edelstein, Amouroux, Battesti & Florent (1982). Chemotactic response of peripheral blood monocytes is depressed *in vitro* in about 50% of cancer patients (Boetcher & Leonard, 1974). Moreover, the prognosis of patients with depressed chemotactic activity may be less favorable than that of patients with normal responses (Israel *et al.*, 1982). The brisk inflammatory response elicited by the intraperitoneal administration by *C. parvum* in heavily pretreated ovarian cancer patients suggests, however, that an adequate chemotactic stimulus can evoke migration of both polymorphonuclear and mononuclear leukocytes (Bast *et al.*, 1983).

The administration of tumor specific monoclonal antibodies which possess chemotactic activity might enhance tumoricidal host defense mechanisms by directing the migration of inflammatory cells to a tumor site. Additional studies are required to assure that monocytes, once attracted to the tumor site, are sufficiently activated for tumor cell killing either alone or in the presence of non-conjugated antibodies. Work with clonogenic assays suggests that macrophages might stimulate as well as inhibit tumor growth (Salmon & Hamberger, 1978). Direct contact with large numbers of activated macrophages generally inhibits rather than stimulates tumor cell growth *in vitro*. If mononuclear cells attracted by antibody-fMLP conjugates are incapable of mediating tumor cell killing, additional intervention may be required. *Corynebacterium parvum* and interferon are capable of augmenting macrophage mediated cytotoxicity and both agents could be administered intraperitoneally. Novel conjugates might also be prepared between specific monoclonal immunoglobulins and other agents known to activate macrophages. Given large amounts of these extraordinarily homogenous immunoglobulins with predictable specificity appropriate clinical trials should be feasible.

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